



Europäisches Patentamt
European Patent Office
Office européen des brevets



Publication number:

0 422 241 A1

(12)

EUROPEAN PATENT APPLICATION
published in accordance with Art.
158(3) EPC

(21) Application number: **90905648.3**

(22) Date of filing: **30.03.90**

(86) International application number:
PCT/JP90/00432

(87) International publication number:
WO 90/11823 (18.10.90 90/24)

(51) Int. Cl.⁵: **B01F 17/14, B01F 17/38,**
B01F 17/56, C07F 9/09,
C12P 9/00, C12P 19/00,
C07H 11/04, //A23J7/00

(30) Priority: **13.04.89 JP 91840/89**

(43) Date of publication of application:
17.04.91 Bulletin 91/16

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB IT LI LU NL SE

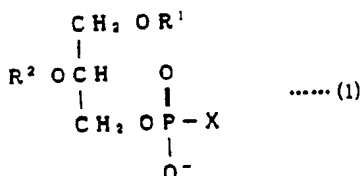
(71) Applicant: **KABUSHIKI KAISHA YAKULT**
HONSHA
1-19, Higashishinbashi 1-chome
Minato-ku Tokyo 105(JP)

(72) Inventor: **KUDO, Satoshi, c/o K.K. Yakult**
Honsha
1-19, Higashishinbashi 1-chome, Minato-ku
Tokyo 100(JP)
Inventor: **NISHI, Eriko, c/o K.K. Yakult Honsha**
1-19, Higashishinbashi 1-chome, Minato-ku
Tokyo 100(JP)

(74) Representative: **Warcoin, Jacques et al**
Cabinet Régimbeau 26, avenue Kléber
F-75116 Paris(FR)

(54) **SURFACTANT AND PROCESS FOR PRODUCING THE SAME.**

(57) A surfactant containing at least one lysophospholipid represented by general formula (1), wherein R¹ and R² each represents a hydrogen atom or an aliphatic acyl residue, provided that one of them is a hydrogen atom and the other is an acyl group; and X represents an organic residue formed by removing a hydrogen atom from an arbitrarily selected hydroxy group of a polyhydric alcohol.



EP 0 422 241 A1

SURFACTANT AND METHOD FOR PRODUCING THE SAME

Field of the Invention

The present invention relates to a surfactant and the method for producing the same.

5

Background Art

There have been generally used soybean lecithin, sucrose fatty acid ester, glycerin fatty acid ester, sorbitan fatty acid ester, propylene glycol fatty acid ester as the food surfactant, conventionally. Among
 10 them, soybean lecithin has been modified for the objective specifically to improve the HLB value thereof. That is, although soybean lecithin is excellent in terms of safety, its HLB value is as low as 3 to 8, which results in the disadvantages not only that the lecithin is difficult to be dissolved in water but that its surfacting action is exhibited only within a limited range, involving the limited use thereof. For that reason, investigation has been carried out over the modification of soybean lecithin in order to provide it with an
 15 HLB value approximately at the same degree of that of other higher HLB emulsifiers.

Soybean lecithin has been modified by a variety of means including transphosphatidylation (Japanese Patent Laid-open No. 61-199749), acetylation, hydroxylation, hydrolysis of fatty acid moiety, owing to the easy modifiable molecular structure thereof. Consequently, transfer lecithin is in practical use on confirmation that transfer lecithin in which the choline bonded with the phosphoryl group of lecithin is substituted
 20 with polyhydric alcohol such as glycerol, glucose, sorbitol and the like, has an HLB value of about 15.

However, some other surfactants produced by chemical synthesis, for example the sucrose fatty acid ester in which the monoester content is increased to 95% or more, have an HLB value of nearly 20, and they are popularly used in various fields due to the excellent and unique surfacting action owing to the high HLB thereof.

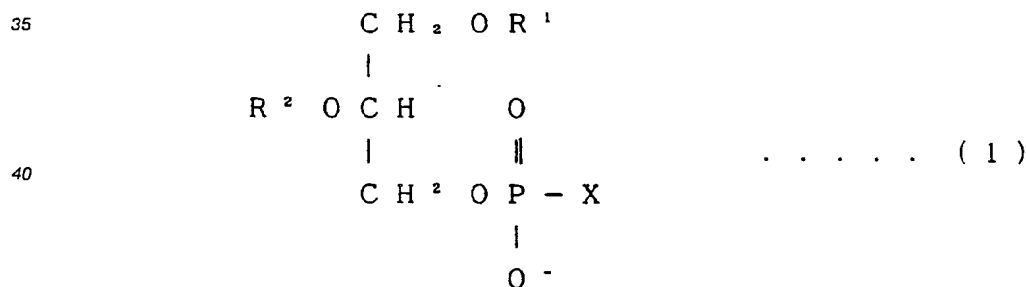
25

Disclosure of the Invention

The present invention is to provide a lecithin surfactant superior to synthetic products in terms of safety,
 30 as one which is ready to use due to the HLB value higher than the conventional products and which has more remarkable surfactant activity.

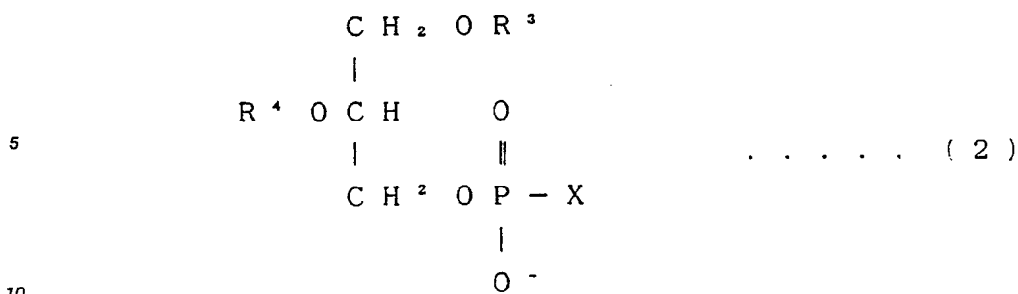
The present invention successfully achieving the above objective is to provide a surfactant containing one or more of lysophospholipid represented by general formula;

35



45

[wherein R¹ and R² represent hydrogen atom or the acyl residue of fatty acid, but either one of R¹ and R² is hydrogen atom, the other being acyl group. X represents an organic group remaining after removing one hydrogen atom of an optional hydroxyl group of polyhydric alcohol therefrom]; and to provide a method for producing the surfactant of the present invention, by treating phosphatidic acid derivative represented by
 50



[wherein R³ and R⁴ represent the acyl residue of fatty acid; X represents an organic group remaining after removing one hydrogen atom of an optional hydroxyl group of polyhydric alcohol therefrom], with lipase or phospholipase A₂ for partial hydrolysis.

It is specifically preferable that the surfactant of the present invention is the one wherein the polyhydric alcohol corresponding to the organic group X in the general formulas (1) and (2) is ethyleneglycol, glycerol, sorbitol, mannitol, glucose, galactose, fructose, sucrose or lactose. In other words, the surfactant with the organic group corresponding to them exhibits an HLB as high as 20 or more, readily dissolves in water and exhibits excellent properties superior to those of the conventional lecithin surfactants, in terms of a great number of points such as foaming power, permeating potential, solubilizing potential, emulsifying potential and the like.

In case that the surfactant of the present invention is produced from the phosphatidic acid derivative of the general formula (2), the phosphatidic acid derivative as a material may be produced through transphosphatidylation from natural phospholipids such as soybean lecithin, egg yolk lecithin, etc., and polyhydric alcohol. Phosphatidic acid derivatives commercially available as the product name transfer lecithin, for example, phosphatidylglycerol, may be also used.

Transfer lecithin commonly contains unreacted lecithin at a high ratio, but such transfer lecithin of itself may be used as the material for the producing method according to the present invention.

As phospholipase A₂ to be reacted with a material compound, pancreatin derived from animal pancreas, or phospholipase A₂ of venom and the like may be used. In case of using phospholipase A₂ as the enzyme, the β position of the material phosphatidic acid derivative is hydrolyzed, to generate lysophospholipid having a hydroxyl group at the position.

In case of using lipase as the enzyme, both or either one of α and β positions of the material phosphatidic acid derivative is hydrolyzed. The reaction can be regulated by controlling the reaction condition, and it is also possible to isolate and purify the reaction products.

The multiple reaction products with different hydrolyzed positions, in a mixture, may be possibly used as a surfactant.

In case of using any enzyme, the enzyme may be used in the form of immobilized enzyme.

Suitable amounts of an enzyme and calcium ion for activating the enzyme are added to the material phosphatidic acid derivative previously suspended in water, which is then kept and reacted at about 20 - 60°C. Because the enzyme activity is generally decreased at an acidic condition and it promotes degradation of materials and reaction products at pH too high, a reaction solution is appropriately at pH 7 - 8.5.

Since the pH of a reaction solution is lowered by released fatty acid as the reaction progresses, it is preferable during the reaction to maintain the pH within the range, by dropwise addition of alkali. The progress of the hydrolytic reaction can be estimated from the amount of the alkali added dropwise.

The reaction mixture is heated at an appropriate point to inactivate the enzyme for termination of the reaction. The reaction solution may be used as surfactant as it is, because the reaction material is also a surfactant.

However, the solution can be treated with lyophilization or fractionation to concentrate the principal ingredient thereof, if necessary, whereby a surfactant with a remarkably enhanced activity can be obtained.

Lysophospholipid of the general formula (1), having an HLB value of 20 or more, dissolves in water more easily than known phospholipid surfacting agents, and exceeds them concerning surfacting action. The foaming power, permeating potential, solubilizing potential and the like of lysophospholipid are in the same degree as or higher than those of conventional phospholipid emulsifiers.

Lysophospholipid has excellent emulsifying potential, and concerning D-phase emulsifying potential specifically, it has even more excellent performance property than conventional products. Furthermore, it is remarkable in terms of organoleptic properties such as taste and smell.

Because the principal ingredient has the characteristic features insofar mentioned, the surfactant of the present invention is more easily usable and is excellent with a wider application field, than the conventional phospholipid surfactants.

5

Best Mode for Carrying out the Invention

The present invention will be explained hereinbelow with reference to Examples.

10

Example 1.

Ten grams of transfer lecithin containing 84% by weight of phosphatidylglycerol (referred to as PG80, hereinafter) was suspended in 50g of water, followed by addition of calcium chloride to 10mM, and was adjusted to pH 8.0 with sodium hydroxide, to which was subsequently added pancreatic pancreatin (Sigma Co.) of 2000 units and reacted at 37° C overnight. An aqueous solution of sodium hydroxide was added dropwise during the reaction so that the solution might be kept at pH 8. Then, the obtained reaction solution was centrifuged and the calcium salt of the fatty acid released during the reaction was removed off as a precipitation.

The supernatant contained 89.0% by weight of lysophosphatidylglycerol where the solid concentration was 11.0% by weight and the β position of the solids was hydrolyzed. This was subjected as a surfactant to the following performance evaluation test. Example 2.

Following the same as in Example 1, there was produced a surfactant containing about 60% by weight of lysophosphatidylglycerol where the solid concentration was 12.0% by weight and the β position of the solids was hydrolyzed, from transfer lecithin containing about 30% by weight of phosphatidylglycerol (referred to as PG30, hereinafter).

Example 3.

30

Following the same as in Example 1, except that the lipase from *Rhizopus deremer* (Seikagaku Kogyo K.K.) was used as enzyme instead of pancreatin and that the pH of the reaction solution was modified to 7.0, the hydrolysis and subsequent treatment was done. The purified reaction product contained lysophosphatidylglycerol hydrolyzed at α position and lysophosphatidylglycerol hydrolyzed at β position.

Examples 4 - 10.

Using 10g of the following transfer lecithins (each with a purity of 80% or more) as a starting material in place of the transfer lecithin (phosphatidylglycerol) in Example 1 and following the same concerning the remaining parts, surfactants composed of their corresponding lyso-type derivatives were produced.

40

<u>Example</u>	<u>Starting material</u>
4	Phosphatidylethyleneglycol
5	Phosphatidylsorbitol
6	Phosphatidylmannitol
7	Phosphatidylglucose
8	Phosphatidylgalactose
9	Phosphatidylfructose
10	Phosphatidylsucrose

55

Examples of Performance Evaluation Test

The surfactants of Examples 1 to 3, the transfer lecithins PG80 and PG30 as their materials, and the surfactants of Examples 4 to 10, were measured of their HLB, according to the actual measurement of emulsification. The surfactants of Examples 1 to 3 were also subjected to the evaluation test of surfacting action. For comparison, the same test was performed on commercially available surfactants. The added amount of the products of Examples was the added amount converted to solids. The surfactants used in Comparative Examples were as follows;

- Comparative Example 1: Polyglycerin fatty acid ester MSW 750 (Sakamoto Pharmaceutical Industry; HLB:13)
- Comparative Example 2: Sucrose fatty acid ester F 160 (Daiichi Kogyo Pharmaceuticals; HLB:15)
- Comparative Example 3: Neutral Detergent Mild (Tamanohada Soap)
- Comparative Example 4: Polyglycerin fatty acid ester DK ester SS (Sakamoto Pharmaceutical Industry; HLB:19)
- Comparative Example 5: Polyglycerin fatty acid ester ML 500 (Sakamoto Pharmaceutical Industry; HLB:13)
- Comparative Example 6: Polyglycerin fatty acid ester ML 750 (Sakamoto Pharmaceutical Industry; HLB:15)
- Comparative Example 7: Soybean lecithin (Ajinomoto; HLB:6)
- Comparative Example 8: Lyso-type lecithin (mixture of lysophosphatidylcholine and phosphatidylethanolamine; Kyowa Fermentation; HLB:12)
- Comparative Example 9: Fractionated phosphatidylcholine PC 80 (Uni-Mills; HLB:7)

1. HLB

The results of measurement are shown in Table 1.

Table 1.

	<u>Surfactant</u>	<u>HLB</u>
	PG 80	17.3
	PG 30	13.0
	Example 1	25.0
	Example 2	22.1
	Example 3	23.0
	Example 4	22.0
	Example 5	29.3
	Example 6	29.1
	Example 7	28.5
	Example 8	29.8
	Example 9	27.0
	Example 10	30.2

2. Permeating potential

Canvas of a 2cm diameter (Canvas Disk #6) is gently placed on an aqueous sample solution of 0.25% concentration.

5 When the canvas gets wet to purge air from the cloth, the disk sinks. The time required to make the disk sink below the water surface is measured to indicate the permeating potential (The shorter the time required until the precipitation, the more intense the permeating potential is.

In case that the precipitation time is within 20 minutes, the measurement is repeated five times, thereby determining a mean value of three measured values excluding the longest and the shortest ones. If the time is 20 min - 30 min, determine a mean value of two measured values. The time 30 minutes or more is not measured.)

The results of measurement are shown in Table 2.

Table 2.

<u>Surfactant</u>	<u>Time until precipitation</u>
Comparative Example 1	30 min or more
20 Comparative Example 3	10 min 39 sec
Comparative Example 4	30 min or more
25 Comparative Example 5	5 min
Comparative Example 6	4 min 55 sec
Comparative Example 7	30 min or more
30 Comparative Example 8	8 min 52 sec
Comparative Example 9	30 min or more
35 PG 80	25 min 35 sec
PG 30	21 min 14 sec
40 Example 1	2 min 37 sec
Example 2	3 min 50 sec
45 Example 3	3 min 33 sec

3. Foaming power

50 An aqueous 0.2% sample solution of 5ml is placed in a tube with screw cap and is stirred at 67 times/min for five minutes. The height of foam is measured, immediately and 1 hour after.

The results of measurement are shown in Table 3.

55

Table 3.

<u>Surfactant</u>	<u>Immediately</u>	<u>One hour</u>
	<u>after stirring</u>	<u>after stirring</u>
Comparative Example 2	13	12
Comparative Example 6	41	38
PG 80	21	13
PG 30	3	--
Example 1	36	34
Example 2	3	--
Example 3	29	20

4. D-phase emulsifying potential (emulsification of O/D type)

When an emulsifying agent is dissolved in polyhydric alcohol at a high concentration and oil is then added, the oil may eventually be incorporated in the state of transparent solution. When the transparent solution is introduced into water, the oil is dispersed in water after being turned into extremely fine particles, whereby a stable oil-in-water emulsion can be easily obtained without using mechanical power. This is D-phase emulsion. Quillaia saponin is remarkable in terms of D-phase emulsifying potential and is in practical use for emulsifying perfume.

Regarding the products of Examples and quillaia saponin Quillaianin C-100 (Maruzen Kasei K.K.), the D-phase emulsifying potential in their formulas hereinbelow described was investigated.

Table 4.

Material	Formula			
	A	B	C	D
Emulsifying agent	0.08	0.08	0.10	0.50
Isomerized sugar (Bx73.6)	17	---	64	64
Water	1.5	2	2	---
Sorbit (75%)	---	17	---	---
Rapeseed oil	55	56	---	---
Orange oil	---	---	20	20

The results of the test are as follows:

Formula A: Only Example 1 succeeded in D-phase emulsion.

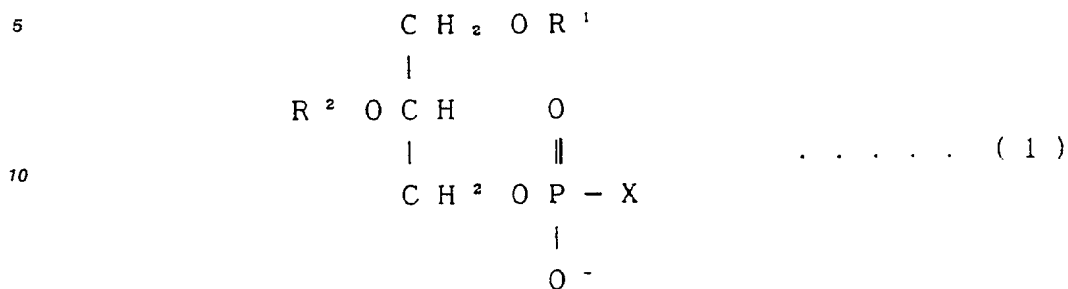
Formula B: Only Example 1 succeeded in D-phase emulsion.

Formula C: Only Example 1 succeeded in D-phase emulsion.

Formula D: Examples 1 to 3 and Quillaianin C-100 succeeded in D-phase emulsion.

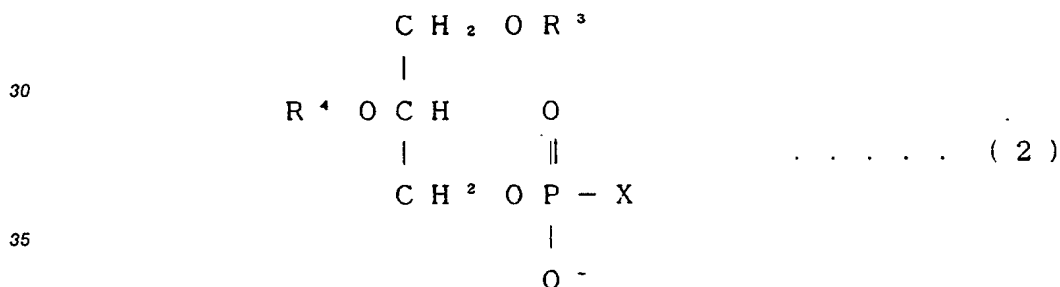
Claims

1. Surfactant containing one or more of lysophospholipid represented by general formula;



[wherein R¹ and R² represent hydrogen atom or the acyl residue of fatty acid, but either one of R¹ and R² is hydrogen atom, the other being acyl group; X represents an organic group remaining after removing one hydrogen atom of an optional hydroxyl group of polyhydric alcohol therefrom].

2. The surfactant according to claim 1, wherein polyhydric alcohol comprises one kind or several kinds selected from ethyleneglycol, glycerol, sorbitol, mannitol, glucose, galactose, fructose, sucrose and lactose.
3. Method for producing a surfactant, comprising treating phosphatidic acid derivative represented by



[wherein R³ and R⁴ represent the acyl residue of fatty acid; X represents an organic group remaining after removing one hydrogen atom of an optional hydroxyl group of polyhydric alcohol therefrom], with lipase or phospholipase A₂ for partial hydrolysis.

4. The method for producing a surfactant according to claim 3, wherein polyhydric alcohol comprises one kind or several kinds selected from ethyleneglycol, glycerol, sorbitol, mannitol, glucose, galactose fructose, sucrose and lactose.
5. The method for producing a surfactant according to claim 3 or 4, wherein the phosphatidic acid derivative comprises a material transfer lecithin in which the base bonded with a phosphoryl group of natural phospholipid is substituted with the polyhydric alcohol.
6. The method for producing a surfactant according to claim 3, wherein the phospholipase A₂ is pancreatin derived from animal pancreas and/or phospholipase A₂ of venom and the like.
7. The method for producing a surfactant according to any one of claims 3 to 6, wherein the phosphatidic acid derivative is hydrolyzed at β position by said phospholipase A₂.
8. The method for producing a surfactant according to any one of claims 3 to 5, wherein the phosphatidic acid derivative is hydrolyzed at α or/and β positions with lipase.

9. The method for producing a surfactant according to claim 3, wherein the reaction solution in the hydrolysis of said phosphatidic acid derivative is at pH 7 to 8.5.
10. The method for producing a surfactant according to claim 3, comprising adding an appropriate amount
5 of calcium ion to the reaction solution in the hydrolysis of said phosphatidic acid derivative.

10

15

20

25

30

35

40

45

50

55

INTERNATIONAL SEARCH REPORT

International Application No. PCT/JP90/00432

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int. Cl ⁵	B01F17/14, 17/38, 17/56, C07F9/09, C12P9/00, 19/00, C07H11/04//A23J7/00	
II. FIELDS SEARCHED		
Minimum Documentation Searched :		
Classification System	Classification Symbols	
IPC	B01F17/14, 17/38, 17/56, B01J13/00-13/02, C07F9/09-9/10, A23J7/00, A61K7/00, C07H11/04, C12P9/00, 19/00	
Documentation Searched other than Minimum Documentation to the extent that such documents are included in the fields searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
X	JP, A, 61-88886 (Meito Sangyo Co., Ltd.), 7 May 1986 (07. 05. 86), (Family: none)	1 - 2
Y	JP, A, 61-88886 (Meito Sangyo Co., Ltd.), 7 May 1986 (07. 05. 86), (Family: none)	3 - 10
Y	JP, A, 58-51853 (Kyowa Hakko Kogyo Co., Ltd.), 26 March 1983 (26. 03. 83), (Family: none)	1 - 10
Y	JP, A, 63-245684 (Shokuhin Sangyo Biochemical Reactor System Gijutsu Kenkyu Kumiai), 12 October 1988 (12. 10. 88) & EP, A2, 285421	1 - 10
Y	JP, A, 63-279753 (Taiyo Kogaku K.K.), 16 November 1988 (16. 11. 88), (Family: none)	1 - 10
<p>* Special categories of cited documents: **</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
June 20, 1990 (20. 06. 90)	July 2, 1990 (02. 07. 90)	
International Searching Authority	Signature of Authorized Officer	
Japanese Patent Office		

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	JP, A, 63-42691 (Showa Sangyo K.K.), 23 February 1988 (23. 02. 88), (Family: none)	1 - 10
Y	JP, A, 63-91306 (Asahi Denka Kogyo K.K.), 22 April 1988 (22. 04. 88), (Family: none)	1 - 10
Y	JP, A, 62-205788 (Shoichi Shimizu, Tsuneo Yamane), 10 September 1987 (10. 09. 87), (Family: none)	1 - 10

☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers, because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim numbers, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim numbers, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y JP, A, 62-262998 (Q.P. Corp.),
16 November 1987 (16. 11. 87),
(Family: none)

1 - 10

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.